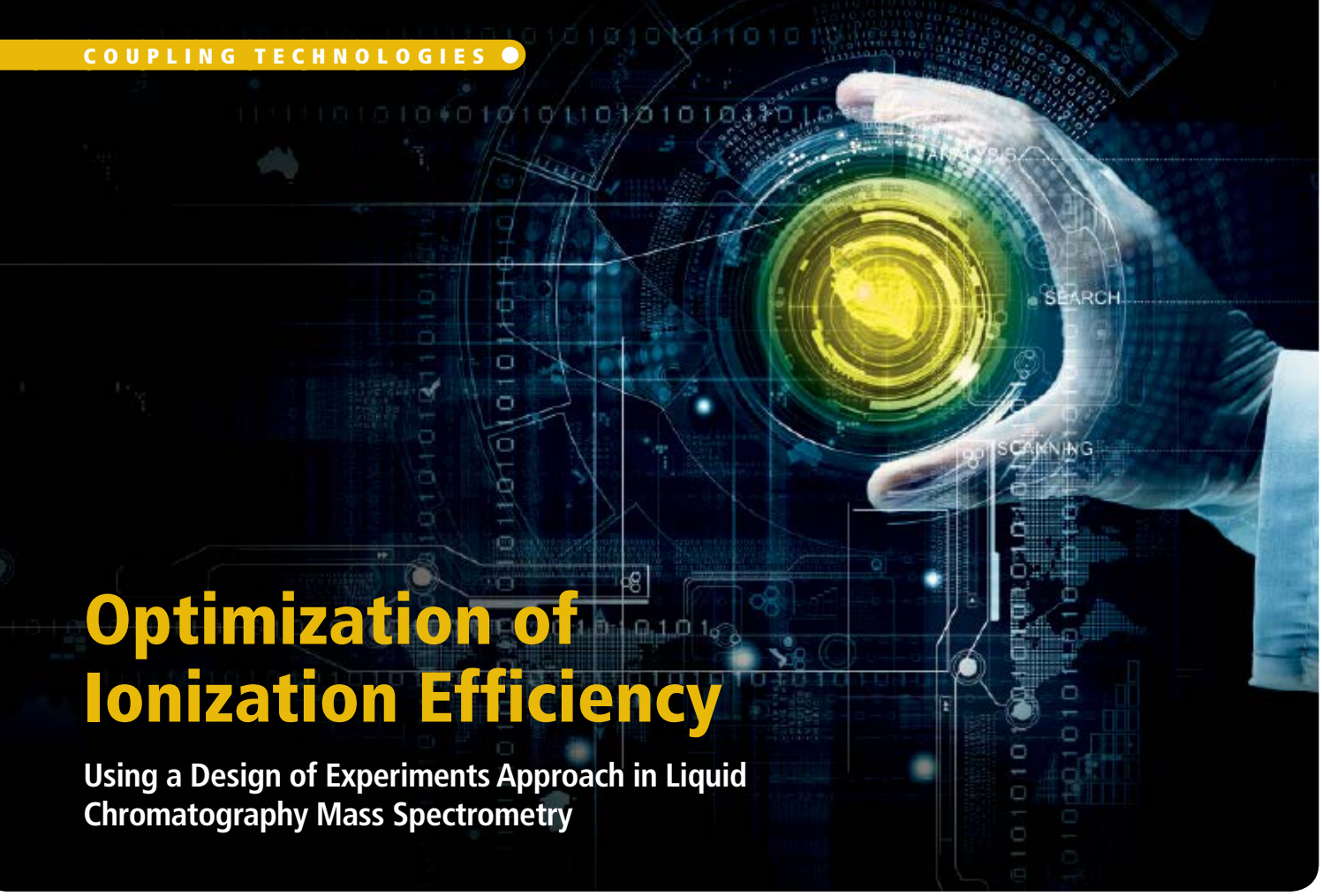

Optimization of Ionization Efficiency

Using a Design of Experiments Approach in Liquid Chromatography Mass Spectrometry

Liquid chromatography hyphenated to mass spectrometry (LC/MS) has become the method of choice in nearly all areas of life sciences, because mass spectrometry is ideally suited to detect and quantify a high number of compounds within one chromatographic run. Moreover, low limits of detection can be obtained which is of utmost importance in environmental and residue analysis. In order to precisely quantify compounds in ultra-trace levels, e.g. in the pg L^{-1} range, the ionization efficiency for target analytes must be very high. Therefore, a careful optimization of the ion source parameters has to be done. Usually, this process is governed by trial and error or using a software algorithm from the MS manufacturer. In this article we describe an alternative approach to optimization of ionization efficiency based on Quality by Design (QbD) principles utilizing Design of Experiments (DOE) methodology.

Method Development in Liquid Chromatography Mass Spectrometry

Method development can be done to optimize the chromatographic method as well as the mass spectrometric settings. There are a number of commercially available software tools which can be used for computer aided method development in liquid chromatography such as ChromSword, DryLab, Fusion QbD, LC & GC Simulator, and Osiris. Although a chromatographic separation is often not mandatory when using a mass spectrometer, isobaric compounds that cannot be differentiated by their mass-to-charge (*m/z*) ratio have to be separated chromatographically [1]. Afterwards, the MS settings need to be adjusted. This can be done by flow injection analysis (FIA) to tune the voltage, gas pressures, temperature and other important parameters. Usually, an algorithm of the MS software is able to automatically run a predefined set of experiments. This means that specific values for each parameter that exert an influence on the ionization efficiency of the analytes and the evaporation of the mobile phase must be defined. The ionization of analytes is influenced by the ion source temperature (TEM), the ionization voltage (IS), the source gas 1 (GS1), and the source gas 2 (GS2). The transfer of the ionized compounds into the vacuum area of the mass spectrometer (MS) and separation from non-ionized ingredients can be controlled by the curtain gas (CUR). Another important parameter for triple quadrupole instruments operated in multiple reaction monitoring mode (MRM) is the collision activated dissociation (CAD) gas. Once the user has defined specific values for all parameters, the MS software algorithm automatically varies all parameters and records the obtained signal intensity for each analyte. At the end, the software recommends a method that is based on the highest total number of counts over all analytes.

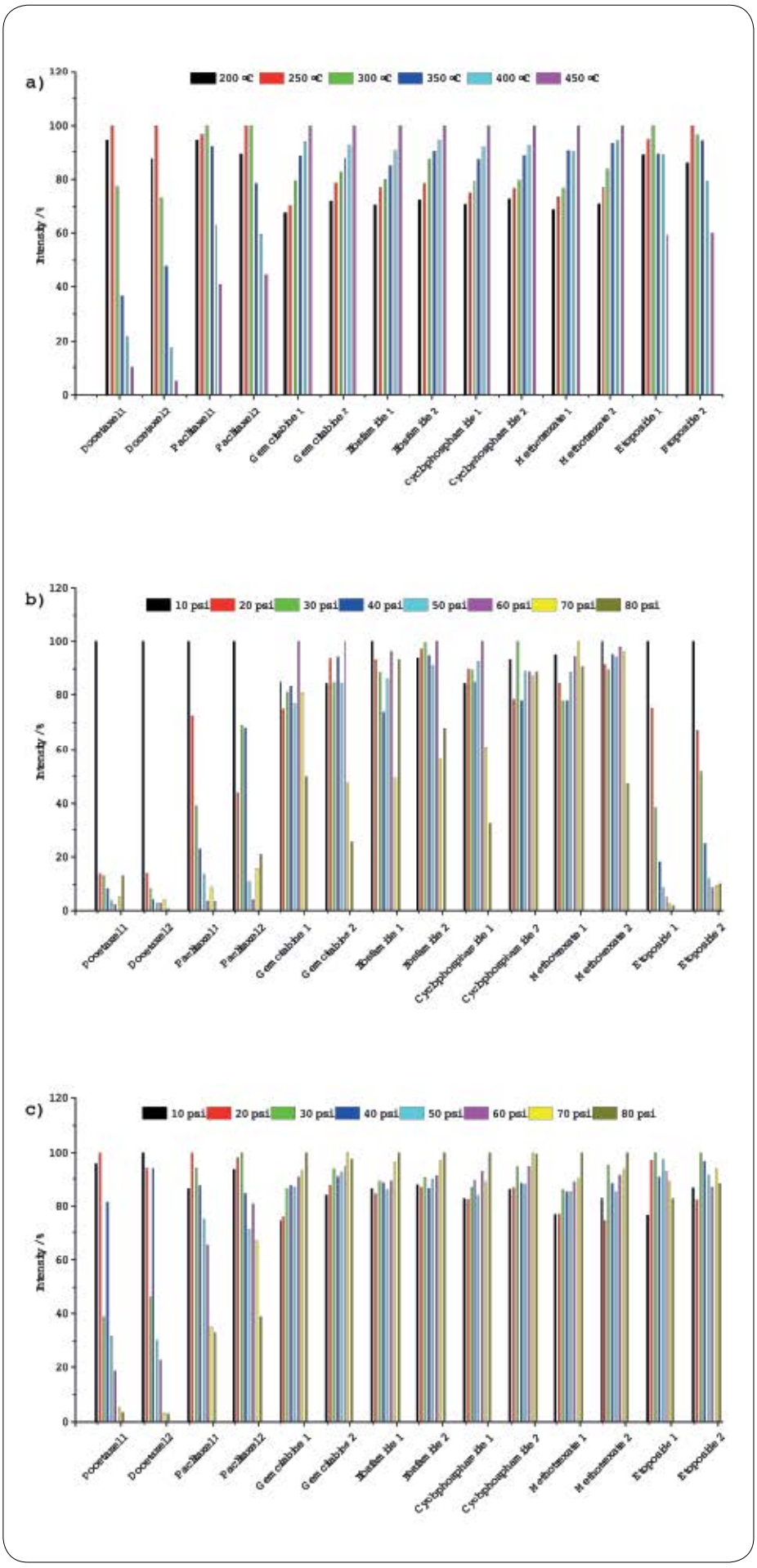

Fig 1: Influences of TEM and GS2 on the intensities of the individual compounds. Two mass transitions are given for each compound. All results are normalized to the maximum observed intensity.

Another procedure is to use a QbD approach. In order to successfully apply such software, only boundary conditions need to be defined and not specific values for each parameter (CUR, CAD, IS, TEM, GS1, GS2). The software then creates a list of experiments based on the combinations of conditions required to support statistical modelling of the parameter effects. After running all experiments, the resulting data is entered into the ObD software. The software then models the data, and connects the models to a solution search algorithm and a graphical Design Space visualization toolset which enables the user to search for all workable combinations of the source parameters, and also "weight" the QbD search to account for analytes that have a significantly lower intensity at higher temperatures. The following discussion illustrates the difference between the classic approach usually embedded in the MS software and a QbD aligned approach using the Fusion QbD software platform.

Results and Discussion

Table 1 shows the final ion source parameter settings obtained using the classical approach and the QbD approach. As the table data shows, there are pronounced differences in the resulting TEM and GS2 settings between the two approaches. The source temperature is a very critical parameter because it can induce degradation of temperature labile compounds. Therefore, the ability to obtain optimum detector performance at a lower temperature provided by the QbD approach is an extremely important and valuable result.

In order to understand the results it is necessary to visualize the data obtained by the classic MS optimization. The data in figure 1 show the influences of TEM and GS2 on signal intensity for two selected mass transitions for each substance using the classic approach. The intensity is normalized to the highest number of counts to visualize the gain or loss of intensity when varying the parameter. As can be seen from Figure 1a, the ionization efficiency for most analytes is highest at a temperature of 450 °C. This would therefore be the recommended ionization temperature obtained from the MS software. For

Tab 1: Comparison of ion source parameter settings based on the applied evaluation strategy.

	Classical Approach	QbD Approach
Curtain Gas / psi	20	20
CAD-Gas / psi	High	Medium
Ionization voltage / kV	5.5	5.5
Temperature / °C	450	263
Gas stream 1 / psi	20	10
Gas stream 2 / psi	60	28.5

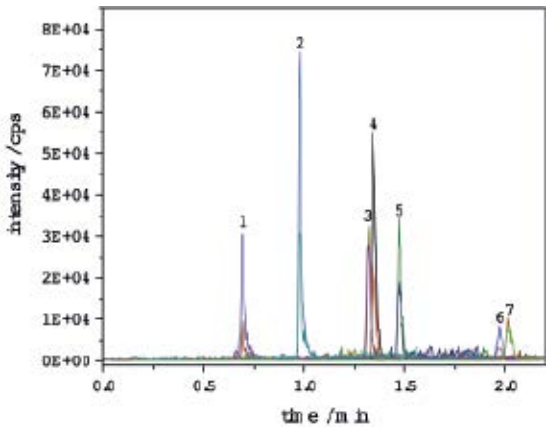

Fig 2: Separation of seven antineoplastic drugs (c: 0.1 ng mL-1). Chromatographic parameters: Stationary phase: YMC Triart C18 (50 x 0.3 µm); Mobile Phase: A: water + 0.1 % formic acid, B: acetonitrile + 0.1 % formic acid; Temperature: 40 °C. Mass spectrometric parameters were optimized by a QbD approach and are given in Table 1. Analytes: 1: Gemcitabine, 2: Methotrexate, 3: Ifosfamide, 4: Cyclophosphamide, 5: Etoposide, 6: Docetaxel, 7: Paclitaxel.

docetaxel, paclitaxel and etoposide, however, a lower temperature of 250 °C is more favourable. At a temperature of 450 °C, the intensity for these substances decreases to less than 10%. If the user pays no attention to such data evaluation, the required limit of detection will not be achieved. In addition, GS2 must be considered. The reason is that a higher gas stream leads to a faster temperature equilibrium in the ion source. The influence of GS2 on the ionization efficiency is highlighted in Figures 1b and 1c for temperatures of 450 °C and 250 °C, respectively. For example, a lower gas pressure of 10 psi is better for all compounds that show a lower intensity at higher temperatures if TEM is adjusted to 450 °C. In contrast, GS2 can be increased to 20 psi if TEM is adjusted to 250 °C. These results are directly obtained using the model-generated graphical visualizations obtained with the QbD approach (tab. 1). Here, an optimum temperature of 263 °C and a gas pressure of 28.5 psi have been suggested in order to account for a higher intensity of docetaxel and paclitaxel. On the basis of these parameters, an optimized MS method for seven antineoplastic drugs can be created. The requirement is that all mass transitions can be detected with a signal-to-noise ratio above three for the lowest calibration standard. The resulting chromatogram is shown in figure 2. As can be seen, the intensity of all target analytes is sufficient. Such a sensitive method cannot be achieved using the results of the classic MS software optimization due to the loss of signal intensity of docetaxel and paclitaxel at 450 °C.

Conclusion

The use of Fusion QbD software to optimize the mass spectrometric settings has been shown to be very efficient. Although most software packages are mainly focused on optimizing the chromatographic method, MS settings are equally important when very low limits of detection must be achieved.

Acknowledgements

We would like to thank Richard Verseput of S-Matrix Corporation for providing the Fusion QbD software. Moreover, we would like to thank Norbert Wenkel for his kind support.

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